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EXPERIMENTAL EVIDENCE OF SEXUAL ISOLATION BETWEEN THREE FORMS OF MICE OF THE CENOSPECIES *PEROMYSCUS MANICULATUS*

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INTRODUCTION

THIS report deals with an experimental test for sexual, or psychological, isolation between three forms of the *Peromyscus maniculatus* species group. A method is described by which the sexual isolation and social behavior of mammals may be studied experimentally. The term sexual or psychological isolation is used here in the sense that it is used by Dobzhansky (1941: 257). It is assumed that copulation between individuals of sexually isolated species fails to occur because of the lack of mutual attraction.

Sexual isolation is a highly important factor in speciation. Once sexual isolation has been achieved between two geographically isolated populations it acts as a barrier to interbreeding that probably can be surmounted only with difficulty if the two populations again come to have overlapping ranges. The stage at which sexual isolation develops is an important one in the differentiation of biological kinds. Some biologists (Dobzhansky, 1937 and 1941; Mayr, 1940 and 1942; and others) believe that the attainment of sexual isolation is the most important event in the differentiation of a species. W. F. Blair (1943) believes that the acquirement of sexual isolation is second only to the development of intersterility as an important stage in differentiation.

No previous attempt to study experimentally the degree of sexual isolation of different kinds of wild mammals is known to us. Dice (1940 and 1942) reasons that some sort of psychological isolating mechanism must operate to prevent the interbreeding of mammals that in the laboratory will hybridize and produce at least some fertile offspring, but which in the field maintain discrete breeding arrays where their ranges overlap.

Sexual isolation has been demonstrated experimentally in *Drosophila* (Sturtevant, 1920; Dobzhansky and Koller, 1938; and Stalker, 1942) and in *Bufo* (A. P. Blair, 1942). Our methods are similar to those of previous workers in that a choice of mating partners is offered the captive animals. The relatively complex social behavior of mammals, however, requires a special technique. With such highly organized forms, the social behavior as well as actual copulation is indicative of the presence or absence of psychological isolating mechanisms.

The experiments were planned by us jointly, and the daily records were made by one or both of us. The report has been written by the senior author, as the junior author was called into military service before the experiments were completed. We are indebted to Charles W. Cotterman for suggestions concerning the statistical treatment of the data and to Carl L. Hubbs for critically reading the manuscript. In the absence of both authors in army service the manuscript has been supervised through the press by Lee R. Dice.

METHODS

Our method of investigation consisted simply of creating an artificial society in a group of laboratory cages. Within this society each mouse could associate with an individual of the opposite sex of its own kind or with either of a pair of mice of a different kind kept in the same group of cages.

Experimental Conditions

CAGES.—Four mouse cages of wire screen, each with floor dimensions of 9 by 16 inches, were connected through the sides by means of short, wooden tunnels. Every mouse could move freely into any cage of the four. Each cage was provided with a food glass, a water bottle, a nest pan, and cotton nesting material. The cages were lettered A to D, to facilitate the keeping of records.

ENVIRONMENTAL CONDITIONS.—The experiments were carried out in a room with northern exposure. No control of temperature was possible during the summer, but during the winter months the room was kept at approximately 72° F.

Breeding condition of the mice.—Only healthy, sexually active mice were tested. It was impossible to use only animals of the same age, but this seemed unnecessary, since all were sexually mature. The breeding condition of these mice can be determined by the condition of the external genitalia. Females going through the estrous cycle have the vagina open, and sexually active males have enlarged testes, located in the scrotum.

Experimental Routine and Treatment of Data

Each experiment was begun by placing four mice, one male and one female of each of the two kinds being tested, in cage A. The mice then were free to distribute themselves through the four cages in any way that suited them. As there were four cages and four nests, each mouse could live in a separate cage and separate nest if it so desired. Each experiment was continued until a litter was born to one of the females, or until a period of approximately forty-five days, which is equal to twice the average gestation period of these mice, had elapsed. Each experimental set was examined daily between eight and ten o'clock in the morning. The mice are active at night and retire to their nests during the day. There was thus obtained a daily record of which mice nested together. As daylight did not come until nearly nine o'clock (Eastern War Time) during the winter months, it was necessary to turn on bright lights in the room for about an hour before all of the mice had retired. When the daily examinations were made the mice were examined for evidence of recent fighting, and the females for possible pregnancy. After an experiment was terminated, the females were segregated and examined frequently until it was evident that they were not pregnant. In order to prevent the carrying over from one experiment to another of any attraction between two individuals, the same pair was never placed together in more than one experiment. Some individuals, however, were reused in a different type of experiment.

The data secured from these experiments are of three types. First, there are the daily records of association in the nests. These may be treated statistically. Second, there are the observations on the fighting that took place in the artificial society made up of two pairs of mice. Third, there is the positive evidence of the choice of mating partners as shown by the paternity of the litters that were born. For most of the litters here reported it is possible to tell the paternity of the young by their appearance. In interpreting the evidence from the production of litters it must be remembered that some of these stocks are only partly fertile with one another, and it is quite possible that pairing and copulation sometimes may take place without the production of offspring. Each of the three kinds of data throws some light on the problem of sexual isolation, and together they afford a measure of the sexual isolation of the mice.

Fifteen different social combinations are possible between the four mice living together in each experiment. These combinations may be grouped according to the type of behavior they indicate. First, and most critical as a test of sexual isolation, are the combinations of one male with one female. Three combinations are possible in which likes (mice of the same type) are paired (see first three columns of social combination scores in Table I). The mice of each kind can be paired simultaneously, or those of either kind can be paired while the other two mice are living singly. We use the word pair to indicate the occurrence together of a male and female of the same stock or strain to the exclusion of the other mice. These three classes of scores will, therefore, be referred to as pair scores. Opposed to the pair scores are the three classes in which a male of one stock or strain has nested with a female of the other type (see last three columns of social combination scores in Table I). These will be referred to as cross-pair scores.

Four combinations are possible in which three mice nest together to the exclusion of the fourth. In referring to these scores, the excluded animal will be spoken of as ''out,'' meaning it is out of the aggregation. Other possible combinations are: (1) males together, females living singly, (2) females together, males living singly, (3) males together in one cage, females together in another, (4) all four mice together, and (5) no two mice together.

In the statistical treatment of the data, the chi-square test of significance has been used. The probability of the occurrence of each social combination on a chance basis is shown at the bottom of each table. In all critical comparisons, however, events of equal probability only are compared, so that a formula for testing a simple 1:1 ratio has been used. There is one degree of freedom. A deviation from the expected equal numbers is considered significant if the chi-square value exceeds 3.841, which marks the 5 per cent level of probability. A deviation is considered highly significant if the chi-square exceeds 6.635, which marks the 1 per cent level of probability.

STOCKS

Origin of the Stocks

Three stocks of mice and the hybrids between them were used in the experiments. The origin of these stocks is as follows:

Leucocephalus, Navarre.—First and second laboratory-born generations from animals that were trapped on Santa Rosa Island, Florida, in the winter and spring of 1942.

Albifrons, Gulf Shores.—Second and third laboratory-born generations from animals trapped on the mainland beaches of Gulf Shores State Park, Alabama, in the winter of 1941–42.

Blandus, Tularosa.—This stock came from the vicinity of Tularosa, Otero County, New Mexico. It has been in the laboratory about four years and has been separated into a number of color strains. Where pairs of *blandus* were used in the experiments only mice of the same color strain were used.

Hybrids.—These were laboratory-produced, F_1 hybrids between *blandus* Tularosa and *leucocephalus* Navarre, and between *blandus* Tularosa and *albifrons* Gulf Shores.

Relationships of the Stocks

The stock of *Peromyscus maniculatus blandus* is representative of the species *maniculatus.* The stocks of *Peromyscus polionotus leucocephalus* and *P. p. albifrons* are representatives of the species *polionotus.* All of these mice belong to the cenospecies *Peromyscus maniculatus* (W. F. Blair, 1943). All will cross in the laboratory if no choice of mating partners is given, but only a small proportion of the species crosses are successful, and in at least the *leucocephalus–blandus* cross some F_1 males are sterile. All of the wild populations represented by these mice are geographically isolated from one another. The population of *leucocephalus* occupies Santa Rosa Island, off the west coast of Florida. The population of *albifrons* occurs on the adjacent mainland and is separated from the nearest representative of the species *maniculatus* by a gap of more than one hundred miles in which neither kind occurs.

THE MATING EXPERIMENTS

Ninety mating experiments were conducted between July, 1942, and February, 1943. In addition to the tests between the different stocks and those between the hybrids and their parent stocks, experiments were carried out in which all mice were from the same stock.

blandus with *blandus*

Sixteen experiments were conducted with *blandus* alone. In these experiments the mice of one pair were of one color strain, whereas those of the other pair were of a different color strain. Four color strains, designated tentatively as dark gray, red, sand, and yellow were used. The sands and yellows differ from the reds and grays in at least one major genetic factor. They are homozygous for a recessive gene for paleness, while most of the reds and dark grays are homozygous for its dominant allele, which produces a dark pelage. The dark and pale mice are conspicuously different in shade. In all of the *blandus* experiments a male and female from one of the pale strains has been tested against a male and a female from one of the dark strains.

The records of the *blandus* with *blandus* tests, except for two that will be discussed later, are summarized in Table I. It is obvious from the summarized daily scores that the distribution of the mice through the four cages was not according to chance. The double-pair and double cross-pair scores and the scores in which the pale mice were paired have a higher frequency than can be attributed to chance. Likewise, the scores in which one or the other male was out of the aggregation and the scores in which all four mice were together exceed the expected frequencies. The frequencies of the scores in which one or the other female was out of the aggregation are very close to the expected. In all of the other classes, the observed frequencies are less than would be expected on the basis of chance.

DOMINANCE AMONG MALES.—*Blandus* is definitely polygamous, at least in a society such as existed in the experiments. One male usually fights the other male, establishes dominance over him, and associates himself with the two females. The dominant male seems to be complete master over the other male. The losing male usually is bitten severely on the tail, sometimes until the tail is completely lost. The rump is also frequently attacked. The hind feet, scrotum, and ears are bitten occasionally. The dominated male is sometimes killed. The dominant male seldom receives so much as a scratch, for the other male usually submits without attempting retaliation.

In nine of the fourteen experiments listed in Table I, one male showed strong dominance by fighting and defeating the other. In the five remaining experiments, also, one male was dominant, but less strikingly so. Two additional experiments, not listed in Table I, had one dominant male in each. In one of these experiments a male was killed on the first night. In the second experiment one male was so completely cowed that he lived in the food glass, consequently the daily scores from this experiment are not comparable statistically with those from the others. In eleven experiments the pale male was dominant, and in five the dark male.

The male that was dominant in fighting also usually associated with the females to the partial exclusion of the other male. This is shown by comparing the daily scores of the dominant with the dominated male. The dominant male was in a nest with both females while the other male was alone a total of 104 times. Only nine times was the dominated male in a nest with the two females while the other male was alone. This deviation from the 1:1 ratio expected by chance is very highly significant, for the chi-square value is 79.9.

The association of the dominant male with the females also may be shown by lumping all scores in which the dominant male was with one or the other or both of the females while the other male was alone, and comparing the result with the lumped scores in which the dominated male was with one or both of the females. The dominant male was with one or both females 186 times, but the other male was with them only forty-three times. The chi-square value for the deviation from the expected 1:1 ratio is 89.3, which is very highly significant.

The pale male was with one or both females a total of 148 times, and the dark male was with the females only eighty-one times. This deviation from the expected 1:1 ratio is highly significant, for the chi-square value is 19.6. The dominance of the pale male was expressed by his frequent pairing with one or the other of the females, not by association with both of them at the same time. There is no significant difference in the latter type scores of the two males. The pale male was with both females to the exclusion of the dark male a total of sixty-five times, and the dark male was with both females forty-eight times. The chi-square value for the deviation from the expected 1:1 ratio is only 2.6, which is not significant.

TABLE I

SOCIAL GROUPS FORMED IN AN EXPERIMENTAL SOCIETY OF *PEROMYSCUS BLANDUS* WITH *BLANDUS*

One pair of pale strain (P) together with one pair of dark strain (D) in each experiment.

Experiment Number	Total Daily Records	Social Combinations															Litters			Fighting	Analysis
		Double Pairs	Dark ♂ with Dark ♀	Pale ♂ with Pale ♀	Dark ♀ Out	Pale ♀ Out	Pale ♂ Out	Dark ♂ Out	None Together	♂♂ Together	♀♀ Together	All 4 Together	♂♂ Together ♀♀ Together	Pale ♂ with Dark ♀	Dark ♂ with Pale ♀	Double Cross Pairs	Likes × Likes	Cross Matings	Father?		
31	39	......	1	......	......	......	2	16	......	......	7	......	5	7	......	1		D × P	P × ?	♂D often bitten ♂P bitten once	One ♂ dominant
39	37	1	1	16	......	......	......	8	5	2	1	......	......	3	......	......			P × ?	♂D often bitten	One ♂ dominant
53	22	2	......	2	......	2	......	11	1	......	2	......	......	2	......	......	P × P(2)			♂D often bitten	One ♂ dominant
61	27	3	7	1	2	2	5	......	......	......	3	2	......	......	2	......	D × D(2)	P × D		♂P often bitten ♀D stole yg. of ♀P	One ♂ dominant
85	52	1	6	3	......	......	26	2	......	1	4	3	......	1	1	4		P × D		♂P often bitten	One ♂ dominant
86	25	2	1	......	3	2	3	7	......	......	1	3	2	......	1	......		D × P		♂D often bitten	One ♂ dominant
54	29	......	......	3	1	1	1	8	1	......	1	1	......	4	2	6	P × P	P × D		♂D often bitten	♂D mated with ♀P, then ♂P dominated
76	48	2	......	......	4	1	11	1	......	......	2	18	1	......	2	6				♂P bitten once	All together, then ♂D dominant
60	27	7	......	6	......	......	......	6	1	......	1	......	......	5	......	1		P × D		One ♂D killed 2d ♂D often bitten	♂P dominant, but ♂D parent of only litter
56	31	2	......	1	6	6	......	2	......	4	1	3	1	......	......	5	P × P(2)	D × P		♂D chased once by a P	♂P sired litters. ♀♀ pregnant and often alone
73	26	......	......	18	1	......	......	......	2	1	......	......	......	......	4	......	D × D(2)			♂P often bitten	♂ and ♀ P paired ♂D dominant
84	29	9	1	4	3	......	......	2	1	1	......	5	......	......	1	2		D × P(2)		♀D often bitten ♂D bitten once	Often paired ♂P dominant
65	30	11	2	5	1	......	......	2	......	......	1	......	......	2	1	5		D × P		♀P and ♂D bitten	Definite pairing and cross pairing
71	24	1	......	......	2	5	......	......	1	2	1	9	1	......	......	2		P × D		♀D bitten twice ♂P killed after litter born	Together, or pregnant ♀P out
Total	446	41	19	59	23	19	48	65	12	11	25	44	10	24	14	32	9	11	2	Frequent fighting	One ♂ dominant
Expected	446	20.9	41.8	41.8	20.9	20.9	20.9	20.9	41.8	41.8	41.8	7.0	20.9	41.8	41.8	20.9					

The dominant males were the fathers of most of the litters conceived during the experiments. Nineteen litters were fathered by dominant males, and only two were sired by nondominant males. This deviation from the expected 1:1 ratio is highly significant, for the chi-square value is 13.8. In both cases in which nondominant males fathered litters the pregnancies took place near the beginning of the experiments and possibly before the dominance of the other male was established. In one of these cases the same female later produced a litter by the dominant male.

Not all of the fighting was restricted to the males. In three experiments one female was bitten a few times, but we do not know which individual did the biting. An interesting item of female behavior was observed in one experiment (No. 61) in which both females bore litters at the same time. The dark female stole the young of the pale female. These young also were dark as they were fathered by a dark male. This female moved the stolen young into her nest and nursed them along with her own offspring. When the experiment was later broken up the stolen young were left with their foster mother, who raised them as well as her own to maturity.

ASSORTATIVE MATING OF COLOR TYPES.—A considerable amount of pairing occurred in the *blandus* experiments, even though one male usually dominated the other. To determine whether or not a nesting partner of the same color strain is selected, the three classes of pair scores may be lumped and compared directly with the sum of the three classes of cross-pair scores.

The total frequency of the pair scores is 119, and that of the cross-pair scores is only seventy. Since the probabilities of the occurrence of either pairing or cross pairing are equal, the chi-square test may be used to test the significance of the deviation from the expected, simple 1:1 ratio. The chi-square value is 12.7, which indicates a highly significant deviation in the direction of the assortative pairing by color strains.

The pale mice were paired more frequently (fifty-nine times) than were the dark mice (nineteen times). Double-pair scores are not included here, because the two types of pairs obviously cancel one another. To include them would unjustifiably multiply the data. The chi-square value for testing the deviation from the expected 1:1 ratio is 20.5, which indicates a highly significant deviation in the direction of pairing of pale with pale.

In actual breeding, as shown by the litters produced, there was no selection by color type by the parents. Twenty-three litters were born. Two died or were eaten before their paternity could be determined. Of the other twenty-one, nine were the result of matings between mice of the same color strain, and twelve were the result of cross matings between strains. There is no significant deviation from an expected 1:1 ratio, for the chi-square value for the deviation is only 0.4.

leucocephalus with *leucocephalus*

Seven experiments were carried out with *leucocephalus* alone. Two males and two females were placed together just as in all the other experiments, but here there were no known genetic differences between any of the mice.

The social behavior of *leucocephalus* is very different from that of *blandus*. There was no evidence of fighting between any of the *leucocephalus* individuals, and all four mice in an experiment usually lived together in the same nest (see Table II). In only one experiment (No. 93) was there any obvious departure from this behavior. In this experiment, the two males frequently nested with the pregnant female while the nonpregnant female was alone. There possibly was some antagonism between the two females.

The least probable combination on the basis of chance (all four mice together) had a frequency greater than the total frequency of all other combinations. In 190 daily records (70.4 per cent of the total) all four of the mice were living together. The observed frequencies of the combinations in which only three mice were together were close to the expected frequencies of those combinations. Also, the observed frequency of the combination in which the males were together in one cage, the females in another, was close to the expected. The frequencies of all other combinations were much below the expected. There was no significant amount of pairing between any two individuals.

Even the birth of a litter did not cause any fighting among the *leucocephalus* individuals. In one experiment (No. 38) both females produced young before they were removed from the experimental cages. After the birth of the second litter, five days after the advent of the first, the two females moved their respective offspring into the same nest. There the two litters became mixed, and each female occasionally nursed young of both litters.

Another habit of undoubted social significance is the storing of food by *leucocephalus*. Sunflower seeds were dropped into the cages once weekly. The mice carefully stored every sunflower seed in one or two of the unused nest pans. The same storage place was used throughout each experiment. If the nest pan was lifted, and the seeds scattered about, they were carefully placed back in the nest pan the following night. No food was stored by *blandus*.

albifrons with *albifrons*

Six experiments were conducted with *albifrons* alone. The results are shown in Table II. The behavior of *albifrons* bore some resemblance to that of *leucocephalus*, but there were obvious differences. There was a tendency for all of the animals in an experiment to nest together, but in only 29.5 per cent of the daily records were all four together, compared with 70.4 per cent for the *leucocephalus*.

Definite pairs were formed and maintained in one experiment (No. 57). This is the only experiment in all of our series in which actual mating behavior was observed. The morning after a litter was born to female A, this female and male A were observed running through the cages. Both mice

TABLE II

SOCIAL GROUPS FORMED IN AN EXPERIMENTAL SOCIETY OF *PEROMYSCUS*

Two males (♂A and ♂B) and two females (♀A and ♀B) of the same form together in each experiment.

Experiment Number	Total Daily Records	Social Combinations															Litters	Fighting	Analysis
		Double Pairs	♂A with ♀A	♂B with ♀B	♀B Out	♀A Out	♂A Out	♂B Out	None Together	♂♂ Together	♀♀ Together	All Together	♂♂ Together ♀♀ Together	♂A with ♀B	♂B with ♀A	Double Cross Pairs			
												leucocephalus with *leucocephalus*							
34	45	1	......	......	......	3	......	1	......	......	......	36	2	......	......	2	....	No evidence of fighting; seeds stored	Lived together amicably
38	31	......	......	......	1	......	......	2	......	......	......	28	......	......	......	......	♀A × ⚲ ♀B × ⚲	No evidence of fighting; seeds stored	Lived together amicably
42	43	......	......	......	3	1	2	3	......	......	1	30	1	1	......	1	....	No evidence of fighting; seeds stored	Lived together amicably
47	48	2	......	......	......	1	2	5	......	......	1	36	1	......	......	......	....	No evidence of fighting; seeds stored	Lived together amicably
67	35	......	......	......	2	1	3	3	......	1	......	25	......	......	......	......	♀B × ⚲(2)	No evidence of fighting; seeds stored	Lived together amicably
92	39	......	......	......	4	2	2	1	......	......	......	27	3	......	......	......	♀B × ⚲(2)	No evidence of fighting; seeds stored	Lived together amicably
93	29	......	......	......	11	......	6	......	......	1	......	8	2	......	1	......	♀A × ⚲	No evidence of fighting; seeds stored	All together or 2 ♂♂ with pregnant ♀A
Total	270	3	......	......	21	8	15	15	......	2	2	190	9	1	1	3	7	No fighting	Strongly gregarious
Expected	270	12.6	25.3	25.3	12.6	12.6	12.6	12.6	25.3	25.3	25.3	4.2	12.6	25.3	25.3	12.6			
												albifrons with *albifrons*							
57	46	14	13	7	1	5	1	......	......	......	2	......	......	2	1	......	♀A × ⚲	♀B bitten once	Definite pairing
51	36	2	2	2	4	3	......	3	1	......	......	14	......	1	1	3	♀A × ⚲(2)	No fighting, stored seeds	Together or temporary pairing
64	29	1	......	1	3	2	1	2	......	......	......	16	1	......	1	1	♀A × ⚲	No fighting evident	Lived together amicably
98	31	......	......	......	3	2	1	4	......	1	2	15	3	......	......	......	....		
117	26	1	1	......	6	2	......	4	......	......	......	7	1	2	......	2	♀B × ⚲	♂B often bitten	♂A dominant
121	25	2	1	......	......	......	10	3	......	......	1	5	1	......	......	2	♀A × ⚲	No fighting evident	♂B dominant
Total	193	20	17	10	17	14	13	16	1	1	5	57	6	5	3	8	6	Some fighting	Moderately gregarious
Expected	193	9.0	18.1	18.1	9.0	9.0	9.0	9.0	18.1	18.1	18.1	3.0	9.0	18.1	18.1	9.0			

were obviously excited, and the male made several attempts to mount the female. The other pair of mice in the experiment were somewhat excited too, but the other male made no attempt to copulate with this female.

One male dominated the other in two experiments (Nos. 117 and 121). In the first of these one male was badly bitten, and in the second, one male was with the females more frequently than was the other. Fighting occurred in two of the six experiments. In addition to the experiment in which a male was badly bitten, there was one in which a female was bitten once. Seeds were stored in only one experiment, and then they were stored only for a short period.

The social behavior of *albifrons* resembles that of *blandus* in the tendency to pair and in the fact that one male sometimes dominates the other. In frequently nesting together the *albifrons* resemble *leucocephalus.* Individuals of *albifrons* are less inclined to store food than are those of *leucocephalus,* and they fight more than do *leucocephalus,* but less than *blandus.* In general, therefore, the behavior of *albifrons* is somewhat intermediate between that of *leucocephalus* and that of *blandus.*

leucocephalus with *blandus*

Seventeen experiments were set up with one pair of *leucocephalus* and one pair of *blandus* (Table III). In each set the male and female of *blandus* were of the same color strain. In eleven of the experiments there was definite pairing by kinds to the exclusion of most other combinations. In two of the others (Nos. 78 and 100) the two males associated with the female *leucocephalus* after the female *blandus* became pregnant. In the four remaining experiments, all four mice were together more frequently than they were in any other combination. The six experiments in which there was little pairing are scarcely less indicative of species discrimination than the experiments in which there was definite pairing. The fact that the males were nesting together a majority of the time in these experiments indicates to us that the psychological differences between the two kinds are so great that little feeling of competition exists between the males.

The frequency of occurrence of double-pair and *leucocephalus*-pair scores is much greater than would be expected on the basis of chance. The frequency of the *blandus*-pair scores is close to the expected frequency. The three classes of cross-pair scores all fall far below the frequencies expected by chance. The combinations of all four mice together and of three together with the *blandus* female out exceed the expected frequencies. The scores of the remaining combinations are close to or less than the expected frequencies.

There was at least a little fighting in eleven of the experiments. The fighting was very different, however, from that in the *blandus* controls, where one male was continually bullied by the other. Here the fighting was sporadic, and one individual was seldom bitten more than once. Females were involved in fighting as much as males; eight individuals of each sex showed evidence of injury from fighting. The fighting was about evenly divided between the forms, for six of the *blandus* and ten of the *leucocephalus* showed evidences of injury. The one exception to the general rule of little fighting occurred in experiment No. 59, in which both *leucocephalus* were killed by the *blandus.*

The sexual isolation between *leucocephalus* and *blandus* is best shown by a comparison of the combined pair scores with the combined cross-pair scores. The pair scores total 394, and the cross-pair scores total only twenty-one. This is a very highly significant deviation from the 1:1 ratio that would be expected in the absence of any discrimination between the two kinds, for the chi-square value is 335.3.

The *blandus* male was with the two females while the other male was alone more frequently than was the *leucocephalus* male. The *blandus* male was with the two females thirty-one times, and the *leucocephalus* male was with them only fifteen times. The chi-square value for testing the deviation from the expected 1:1 ratio is 5.6, which is significant. This probably indicates slight dominance by the *blandus* male. That this dominance, if it is such, is negligible is shown by the fact that the *blandus* male entered into cross pairs no more frequently than did the *leucocephalus* male.

All of the litters born in the experiment that survived until identifications could be made were the result of the mating of likes. Two litters born to *blandus* were killed soon after birth. Nine litters of *blandus* and two litters of *leucocephalus* were identified. Thus, there is no evidence of cross mating under the conditions of the experiment. We cannot rule out the possibility of copulation not followed by pregnancies between mice of the different kinds, because the two kinds are only partly fertile together.

albifrons with *blandus*

Seventeen experiments were conducted with *albifrons* combined with *blandus.* The results are given in Table IV. In five experiments, likes were paired most of the time. In seven experiments, all four of the mice nested together more frequently than they occurred together in any other combination. In two experiments (Nos. 74 and 89) the *blandus* male was dominant. In one experiment (No. 72) the mice were either all four together or cross paired most of the time. In the two remaining experiments (Nos. 83 and 113) the female *blandus* usually was alone, while the two males lived with the female *albifrons.* In one of these the *blandus* female was pregnant, and in the other the female *blandus* possibly bore a litter that was eaten before we found it. Fighting occurred in eleven experiments, the same number as in the *leucocephalus* with *blandus* tests, and in general the fighting was of the same magnitude as in those tests.

Four combinations occurred much more frequently than would be expected on the basis of chance. These were (1) double pairs, (2) all four mice together, (3) *blandus* female out of the aggregation, and (4) *albifrons*

TABLE III

SOCIAL GROUPS FORMED IN AN EXPERIMENTAL SOCIETY OF *PEROMYSCUS*

One pair of *leucocephalus* (L) together with one pair of *blandus* (B) in each experiment.

Experiment Number	Total Daily Records	Social Combinations: Double Pairs	*blandus* ♂, ♀ Together	*leucocephalus* ♂, ♀ Together	*blandus* ♀ Out	*leucocephalus* ♀ Out	*leucocephalus* ♂ Out	*blandus* ♂ Out	None Together	♂♂ Together	♀♀ Together	All Together	♀♀ Together ♂♂ Together	*leucocephalus* ♂ with *blandus*	*blandus* ♂ with *leucocephalus* ♀	Double Cross Pairs	Litters: B×B	L×L	Father ?	Fighting	Analysis
115	26	13	5	3	......	1	......	1	2	1	......	......	......	......	......	......	2			No fighting evident	Definite pairing
33	34	23	4	......	1	......	3	......	......	......	......	......	......	......	3	......			♀B × ?		
79	44	28	8	8	......	......	......	......	......	......	......	......	......	......	......	......				♀ B bitten once	Definite pairing
37	43	7	3	13	2	3	......	1	3	3	......	......	......	6	......	2				No fighting evident	Definite pairing
62	40	32	6	......	......	1	......	......	......	......	......	1	......	......	......	......	2			Both B often bitten	Definite pairing
20	48	18	11	11	1	......	2	......	1	......	1	......	......	1	2	......				♂L wild. ♀L once chased ♀B	Definite pairing
22	42	22	2	16	......	......	......	1	1	......	......	......	......	......	......	......				♂ B, ♀ L bitten once	Definite pairing
23	52	25	12	7	3	......	......	......	......	1	1	......	......	......	3	......	1			♀ L bitten once	Definite pairing
32	42	25	2	15	......	......	......	......	......	......	......	......	......	......	......	......	1			Both L bitten once	Definite pairing
35	45	19	1	18	3	1	......	......	......	2	......	......	......	......	1	......				Two ♀♀ fighting once	Definite pairing
59	6	2	......	2	......	......	2	......	......	......	......	......	......	......	......	......				Both L killed	Definite pairing
78	26	1	......	......	15	2	......	1	......	......	......	6	1	......	......	......			♀B × ?	No fighting evident	After ♀ B pregnant both ♂♂ usually with ♀ L
100	29	2	2	3	13	4	......	......	......	1	1	1	2	......	......	......	1			♀ L bitten once	
87	22	1	......	2	1	1	8	2	......	......	......	7	......	......	......	......	1	1		♀ L bitten once	All together or ♂ L out
29	44	3	......	5	8	5	2	4	......	......	......	15	......	1	......	1				No fighting evident	All usually together or one out
116	26	4	1	1	......	......	8	4	......	......	......	8	......	......	......	......	1	1		No fighting evident	All usually together or one ♂ out
118	30	7	1	......	......	1	6	1	......	......	......	13	......	......	......	1				♀ L bitten once. ♂ B chased ♀ L once	All usually together or paired
Total	599	232	58	104	47	19	31	15	7	8	3	51	3	8	9	4	9	2	2	Occasional fighting	Usually paired
Expected	599	28.1	56.2	56.2	28.1	28.1	28.1	28.1	56.2	56.2	56.2	9.4	28.1	56.2	56.2	28.1					

male out of the aggregation. Three combinations occurred about as frequently as would be expected on a chance basis. These were (1) double cross pairs, (2) *albifrons* female out of the aggregation, and (3) *blandus* male out of the aggregation. All of the other possible combinations occurred much less frequently than would be expected by chance.

Pairing occurred more frequently than did cross pairing. There were 139 instances of pairing compared with sixty-three of cross pairing. The deviation from the 1:1 ratio that would be expected in the absence of species discrimination is highly significant. The chi-square value is 28.6; however, the sexual isolation between *albifrons* and *blandus* is not nearly so great as that between *leucocephalus* and *blandus.* In the tests between *albifrons* and *blandus* 31.1 per cent of all pair and cross-pair scores were cross pairs, and in the tests between *leucocephalus* and *blandus* only 5.1 per cent were cross pairs.

Additional evidence of isolation between these two forms is found in the two experiments in which the two males associated with one female and in the seven experiments in which all four usually nested together. As in the *leucocephalus* with *blandus* test, we believe that the frequent occurrence together of the *albifrons* and *blandus* males indicated that there was relatively little competition between them for mates.

There was considerably more competition between *albifrons* and *blandus* males than there was between *leucocephalus* and *blandus* males. Evidence of competition between males was obvious in two experiments (Nos. 74 and 89). In one, the male *blandus* associated with the females to the exclusion of the *albifrons* male, and in the other the *albifrons* male was killed. Additional evidence that the male *blandus* tended to dominate over the male *albifrons* is in the total daily scores showing association of males and females. The *blandus* male was with both females and the *albifrons* male was alone forty-seven times. The *albifrons* male was with both females and the *blandus* male was alone only twenty times. The chi-square for this deviation from the 1:1 ratio that would be expected in the absence of male dominance is 10.8, which is highly significant.

The *blandus* male was with one or both females while the *albifrons* male was alone a total of 106 times. The *albifrons* male was with one or both females a total of only fifty-one times. The chi-square value for this deviation from the expected 1:1 ratio is 19.3, which is highly significant. These scores indicate that *blandus* males dominate *albifrons* males to a considerable degree under the conditions of the experiment.

All litters that lived long enough to be identified were the result of the mating of like with like. Twenty-one litters were born. Three litters born to *blandus* females and six born to *albifrons* females were killed soon after birth. Eleven litters resulted from the mating of *blandus* with *blandus,* and one was produced by the mating of *albifrons* with *albifrons.* Therefore, all of our evidence indicates that the mice mated only with their own kind. As in the *leucocephalus* with *blandus* test, we cannot be certain that unsuccessful copulation between individuals of the two different forms did not occur.

blandus–leucocephalus Hybrids with *blandus*

Ten experiments were conducted in which F_1 hybrids between *blandus* and *leucocephalus* were tested with *blandus* (Table V). In two of the experiments (Nos. 90 and 107) F_1 *leucocephalus* × *blandus* hybrids were used. In the eight others the reciprocal hybrids were used. The two groups are here treated jointly. In only two experiments (Nos. 63 and 70) was there definite pairing of likes. The hybrid male dominated the *blandus* male in seven experiments, and the *blandus* male was dominant in one experiment (No. 69).

Three combinations were considerably more frequent than could be expected on the basis of chance. These were (1) double pairs, (2) male *blandus* out of the aggregation, and (3) all four mice together. Four combinations were considerably less frequent than could be expected on the basis of chance. These were (1) no mice together, (2) males together, (3) females together, and (4) males together in one cage, females together in another. The scores of the other combinations were fairly close to the expected frequencies.

A comparison of the pair and cross-pair scores shows that there was no isolation between the hybrids and the pure *blandus*. Pairs occurred a total of seventy times, and cross pairs sixty times. This deviation from the expected 1 : 1 ratio is not significant, for the chi-square value is only 0.8. With the production of an F_1 hybrid generation the psychological barrier to the transfer of *leucocephalus* characters to *blandus* is broken down.

Additional evidence of the breakdown of the previously strong sexual isolation is found in the behavior of the males. One male usually was strongly dominant over the other. Just as in the *blandus* controls the dominant male bullied the other and monopolized the company of the females. The hybrid male was strongly dominant in seven experiments, and the *blandus* male was dominant in only one. This dominance by the hybrid males probably is accounted for by the fact that these hybrids are much more vigorous and aggressive than are the pure *blandus*. The dominance of the hybrid males is proved statistically by the scores showing association with the females. The hybrid male nested with both females while the *blandus* male was alone a total of fifty-seven times. The *blandus* male was with both females only seventeen times. The chi-square value for this deviation from the 1 : 1 ratio that would be expected in the absence of male dominance is 21.6, which is highly significant.

The hybrid male nested with one or both females a total of ninety-eight times while the other male was alone. The *blandus* male nested with one or both females only fifty-four times. The chi-square value for this deviation from the expected 1 : 1 ratio is 12.7, which is highly significant. Therefore,

TABLE IV

SOCIAL GROUPS FORMED IN AN EXPERIMENTAL SOCIETY OF *PEROMYSCUS*

One pair of *albifrons* (A) together with one pair of *blandus* (B) in each experiment.

		Social Combinations															Litters				
Experiment Number	Total Daily Records	Double Pairs	*blandus* ♂, ♀ Together	*albifrons* ♂, ♀ Together	*blandus* ♀ Out	*albifrons* ♀ Out	*albifrons* ♂ Out	*blandus* ♂ Out	None Together	♂♂ Together	♀♀ Together	All Together	♀♀ Together ♂♂ Together	*albifrons* ♂ with *blandus* ♀	*blandus* ♂ with *albifrons* ♀	Double Cross Pairs	B × B	A × A	Father ?	Fighting	Analysis
77	26	11	3	......	2	1	......	2	......	2	......	......	......	......	5	......	1		♀A × ?	♀B often bitten ♀A bitten once	Definite pairing
103	28	12	1	10	4	......	......	......	......	......	......	......	......	......	......	1	1			No fighting evident	Definite pairing
112	31	16	5	2	1	......	4	......	......	......	......	1	......	......	2	......	1	1	♀A × ?	♂ and ♀B fighting once	Definite pairing
120	25	17	1	2	......	1	1	......	......	......	1	2	......	......	......	......			♀A × ? ♀B × ?	All bitten	Definite pairing
119	28	11	1	1	1	1	......	......	1	1	......	7	......	1	2	1	1		♀A × ?	♂B bitten once	Definite pairing
72	22	1	1	......	6	2	2	......	......	......	......	5	......	......	......	5			♀A × ?	No fighting evident	More cross pairing than pairing
74	32	1	2	......	......	......	25	......	......	......	......	......	......	......	3	1	2			♂A bitten once	♂B dominant
89	16	1	......	......	1	......	2	1	1	1	1	1	2	......	5	......				♂A killed	♂B dominant
114	18	......	......	......	......	......	2	......	......	......	1	7	2	1	......	5	1			♂ and ♀A bitten once	Lived together or cross paired
80	30	1	2	1	4	2	4	2	......	......	......	7	5	......	1	1	2			No fighting evident	All frequently together
75	31	1	3	1	1	1	1	3	......	......	......	20	......	......	......	......			♀A × ?	No fighting evident	All frequently together
94	54	3	3	1	4	4	......	6	1	......	......	27	1	......	1	3				No fighting evident	All frequently together
95	26	3	1	......	1	2	2	......	......	1	......	14	......	......	1	1	1			No fighting evident	All frequently together
88	61	......	1	......	11	8	1	......	......	1	1	36	......	......	......	2				♀B bitten once	All frequently together
91	22	1	......	1	......	4	......	4	......	......	......	8	......	1	1	2			♀B × ?	♀B dead last day	All frequently together
113	25	3	1	2	13	......	......	......	......	......	......	......	......	......	4	2	1		♀B × ?	♂ and ♀A often bitten ♀B bitten once	Pregnant ♀B usually alone
83	65	4	1	6	20	3	3	2	1	3	......	11	......	1	8	2				♂A bitten once	♀B alone or all mice often together
Total	540	86	26	27	69	29	47	20	4	9	4	146	10	4	33	26	11	1	9	Moderate fighting	Usually paired or all together
Expected	540	25.3	50.6	50.6	25.3	25.3	25.3	25.3	50.6	50.6	50.6	8.4	25.3	50.6	50.6	25.3					

both the indiscriminate pairing and the presence of strong male dominance indicate the absence of sexual isolation between *blandus* and the *blandus–leucocephalus* hybrids.

The parentage of the litters that were produced offers additional evidence of the breakdown of sexual isolation between the *blandus* and the hybrids. Unfortunately, the paternity of the litters could not always be positively determined, because the hybrids were heterozygous for the definitive characters. Three litters, however, positively were the result of matings between *blandus* females and hybrid males. Four litters definitely were produced by matings between males and females of *blandus,* and three were the result of matings between hybrids. Three litters born to *blandus* females and three born to hybrid females were unidentifiable or were lost. The three litters from matings of *blandus* females and hybrid males afford positive evidence of a breakdown of sexual isolation sufficient to allow interbreeding even when there is a choice of mates.

blandus–leucocephalus Hybrids with *leucocephalus*

Nine tests were made between *leucocephalus* and the F_1 hybrids between *leucocephalus* and *blandus* (Table VI). In one experiment (No. 48) F_1 *leucocephalus* × *blandus* hybrids were used. Reciprocal hybrids were used in the others. All are treated jointly. In two experiments, likes were paired together more often than the mice were associated in any other combination. In four experiments, the *leucocephalus* male dominated the hybrid male. In two experiments, all four of the mice were usually together, and in the remaining experiment the two males were usually with the *leucocephalus* female.

Four combinations occurred more frequently than would be expected on the basis of chance. These were (1) double pairs, (2) hybrid female out of the aggregation, (3) hybrid male out of the aggregation, and (4) all four mice together. Four combinations occurred about as frequently as would be expected on the basis of chance. These were (1) *leucocephalus* paired, (2) *leucocephalus* female out of aggregation, (3) *leucocephalus* male out of aggregation, and (4) *leucocephalus* male cross paired with hybrid female. The remaining combinations occurred less frequently than would be expected by chance.

Pair scores occurred much more frequently than did cross pairs. Likes were together a total of 101 times, while cross pairs occurred only thirty-eight times. This deviation from the 1:1 ratio that would be expected in the absence of isolation between the hybrids and the mice of the pure stocks is highly significant, for the chi-square value is 28.6. These results indicate that there is some sexual isolation between *leucocephalus* and the F_1 hybrids.

The *leucocephalus* male usually was more or less dominant over the hybrid male. In the four experiments in which this was most obvious the hybrid male was badly bitten. The *leucocephalus* male was with both females and the hybrid male was alone a total of fifty-one times. The hybrid male was with both females only fifteen times. This departure from the expected 1:1 ratio is highly significant, for the chi-square value is 19.6.

The *leucocephalus* male was with one or both females and the hybrid male was alone a total of one hundred times. The hybrid male, on the other hand, was with one or both females only thirty-nine times. The deviation from the expected 1:1 ratio is highly significant, for the chi-square value is 26.8. Our results show definitely that there was antagonism between the males and that the *leucocephalus* associated with the females more often than did the hybrid male.

Nine litters were produced in this group of experiments. Four were the result of matings between *leucocephalus*. One born to a *leucocephalus* female was lost. Two litters resulted from matings between hybrids. One litter born to a hybrid female was lost, and another was unidentifiable. The number of litters is small, but each of the six that could be identified resulted from the mating of like with like.

blandus–albifrons Hybrids with *blandus*

Only three tests were made between *blandus* and F_1 hybrids between *albifrons* and *blandus*, for only a few of these hybrids were available. In one experiment (No. 102) the hybrids used were F_1 *albifrons* × *blandus*, whereas in the other two the reciprocal hybrids were used. The results are given in Table V. In all three experiments the *blandus* male was somewhat dominant. In one the hybrid male was badly injured, and in another it was badly bitten and finally killed.

The data are too few for statistical treatment, but insofar as they go they indicate that the reaction between *blandus* and *albifrons–blandus* hybrids is essentially like the reaction between *blandus* and the *leucocephalus–blandus* hybrids. There were fourteen records of pairing and seven records of cross pairing. The *blandus* male was dominant, whereas in the test between *blandus* and the *leucocephalus–blandus* hybrids the hybrid male usually dominated. The *blandus* male was with both females and the other male was alone seventeen times, but the hybrid male was with both females only five times. The former male was with one or both females twenty-five times, and the latter was with one or both females only eight times.

One litter, from a mating between *blandus*, was identifiable. Three other litters born to female *blandus* and two born to hybrid females were unidentifiable.

The few data suggest virtual elimination of sexual isolation between *blandus* and the F_1 hybrids, just as in the *leucocephalus–blandus* hybrids.

blandus–albifrons Hybrids with *albifrons*

The results of three tests of *blandus–albifrons* hybrids with *albifrons* are given in Table VI. In one experiment (No. 50) F_1 *albifrons* × *blandus* were

TABLE V

SOCIAL GROUPS FORMED IN AN EXPERIMENTAL SOCIETY OF *PEROMYSCUS*

One pair of *blandus* (B) together with one pair of hybrids (F_1) in each experiment.

		Social Combinations															Litters					
Experiment Number	Total Daily Records	Double Pairs	Hybrid ♂ with Hybrid ♀	*blandus* ♂ with *blandus* ♀	Hybrid ♀ Out	*blandus* ♀ Out	*blandus* ♂ Out	Hybrid ♂ Out	None Together	♂♂ Together	♀♀ Together	All Together	♂♂ Together ♀♀ Together	*blandus* ♂ with Hybrid ♀	Hybrid ♂ with *blandus* ♀	Double Cross Pairs	B × B	$F_1 \times F_1$	B × F_1	Father ?	Fighting	Analysis
blandus with *blandus–leucocephalus* hybrids																						
70	25	12	3	8	......	......	......	......	1	......	1	......	......	......	......	......	1				Both F_1's bitten	Definite pairing
106	24	12	3	......	......	2	......	......	......	......	......	......	......	7	......	......			2		Both F_1's bitten	Definite pairing
63	28	1	2	......	1	......	9	2	......	......	3	2	1	1	2	4				B × ? F_1 × ?	♂B often bitten	F_1♂ dominant
90	26	......	2	......	......	......	10	......	2	......	4	......	......	1	......	3				F_1 × ?	♂B often bitten	F_1♂ dominant
107	30	3	4	......	......	1	13	......	1	......	......	3	2	1	1	1		1		B × ?	♂B often bitten	F_1♂ dominant
82	18	......	......	......	......	......	6	......	1	1	1	......	......	......	7	2				B × ?	♂B often bitten	F_1♂ dominant
105	33	3	2	1	1	......	6	2	3	1	......	1	......	......	7	6		1	1		Both F_1's bitten once Both B often bitten F_1♂ seldom bitten	F_1♂ dominant
97	28	......	......	......	2	......	8	4	......	......	1	9	......	1	......	3		1			No fighting evident	F_1♂ dominant
110	26	3	1	......	4	4	5	......	1	2	......	4	......	......	......	2	1			F_1 × ?	F_1♀ often bitten	F_1♂ dominant
69	30	......	......	10	......	......	......	9	2	......	2	......	......	7	......	......	2				F_1♂ often bitten	♂B dominant
Total	268	34	17	19	8	7	57	17	11	4	12	19	3	18	24	18	4	3	3	6	Frequent fighting	One ♂ usually dominant
Expected	268	12.6	25.1	25.1	12.6	12.6	12.6	12.6	25.1	25.1	25.1	4.2	12.6	25.1	25.1	12.6						
blandus with *blandus–albifrons* hybrids																						
102	20	2	......	4	1	2	3	4	......	......	......	......	2	......	......	2	1				F_1♂ often bitten, finally killed	Pairing and ♂B dominant
111	28	2	1	1	6	4	1	4	......	1	......	7	1	......	......	......				B × ?	F_1♀ often bitten	All together or one out
81	29	1	1	2	1	1	1	9	......	......	1	5	2	1	1	3				B × ? (2) F_1 × ? (2)	F_1♂ often bitten	♂B dominant
Total	77	5	2	7	8	7	5	17	......	1	1	12	5	1	1	5	1	...	...	5	Frequent fighting	One ♂ usually dominant
Expected	77	3.6	7.2	7.2	3.6	3.6	3.6	3.6	7.2	7.2	7.2	1.2	3.6	7.2	7.2	3.6						

used; in the others reciprocals were used. There was definite pairing of likes in one experiment. In the others, all four mice nested together more frequently than they occurred in any other combination.

The data are too few for statistical treatment, but they suggest that the reaction between *albifrons* and the F_1 *blandus–albifrons* hybrids is not greatly different from that between *leucocephalus* and *blandus–leucocephalus* hybrids. Likes were paired twenty-four times, while cross pairs occurred only ten times. The hybrid male associated with the females somewhat more frequently than did the *albifrons* male. The hybrid male was with both females and the *albifrons* male was alone seventeen times, but the *albifrons* male was with the females only eight times. Likewise, the hybrid male was with one or both females twenty-five times, and the *albifrons* male was with one or both only eleven times.

Two litters were born as the result of matings between individuals of *albifrons*. One other litter, born to an *albifrons* female, was lost before it could be identified.

The results suggest that sexual isolation between the hybrids and *albifrons* is greater than that between them and *blandus*.

DISCUSSION

It is shown above that the three forms of mice of the cenospecies *Peromyscus maniculatus* tested differ in their social behavior in an experimental society comprising only two pairs of mice. In a wholly *blandus* society, one male usually fights the other male and establishes dominance over him. The dominant male often associates with the two females to the exclusion of the other male, and he sires most of the young that are produced. The social behavior of *leucocephalus* mice in the experimental society is very different from that of *blandus*. The *leucocephalus* mice are highly social and all four of them usually nested together. These mice are extremely tolerant of one another even when young are in the nest. *Leucocephalus* mice also differ from *blandus* mice in their habit of making community food stores. The behavior of *albifrons* individuals in the experimental society is intermediate between that of *leucocephalus* and that of *blandus*. The *albifrons* mice resemble *leucocephalus* mice in their habit of frequently all nesting together. They also make food stores, but they make them less frequently than do the *leucocephalus* mice. The *albifrons* individuals resemble those of *blandus* in their tendency to form pairs and in the occasional dominance of one male over the other.

Strong sexual isolation between individuals of *leucocephalus* and *blandus* is exhibited in a mixed society made up of these two forms. The two *blandus* usually live by themselves in one nest, and the two *leucocephalus* in a different nest. Only rarely does a male of one stock nest with a female of the other stock. Our evidence indicates that the two kinds of mice do not interbreed under the conditions of the experiment, for all of the litters produced resulted from the mating of *blandus* with *blandus* or of *leucocephalus* with *leucocephalus.* The *blandus* male forms an aggregation with the two females to the exclusion of the other male slightly more often than does the *leucocephalus* male. This possibly indicates slight dominance by the *blandus* male. The deviation, however, from the 1:1 ratio between the two types of aggregations is not highly significant. Occasional fighting occurs in this society, but, unlike the fighting in the pure *blandus* society, it is sporadic; an individual is seldom bitten more than once during an experiment.

Sexual isolation is strong also between *albifrons* and *blandus,* but it is not nearly so strong as that between *leucocephalus* and *blandus.* This difference in the degree of sexual isolation by which the two forms of *Peromyscus polionotus* are separated from *blandus* is shown by the higher percentage of cross-pair scores in the *blandus–albifrons* society and by the fact that the *blandus* male sometimes dominates the *albifrons* male. In the *blandus–albifrons* society 31.1 per cent of all pair and cross-pair scores were cross pairs, whereas in the *blandus–leucocephalus* society only 5.1 per cent were cross pairs. The *blandus* male associated with the females significantly more frequently than did the *albifrons* male. All of the young that lived long enough to be classified resulted from the mating of *blandus* with *blandus* or of *albifrons* with *albifrons.* The F_1 hybrids between *blandus* and *leucocephalus* and between *blandus* and *albifrons* showed no sexual isolation from pure *blandus.* Cross pairs between *blandus* and the hybrids occurred about as often as did pairs between pure *blandus* or between the hybrids. One male dominated the other as strongly as in the pure *blandus* society. The hybrid male usually dominated the *blandus* male, probably because of its greater vigor and aggressiveness. The dominant male fought the other male and monopolized the company of the females. The hybrids and the pure *blandus* interbred even though the *blandus* individuals had the opportunity of breeding with their own kind.

The sexual isolation between the F_1 hybrids and the *leucocephalus* or *albifrons* stock is less strong than that between the two parent stocks. The extent of isolation between the hybrids and the parent *leucocephalus* or *albifrons* stocks is much greater than that between the hybrids and *blandus.* The mice pair with their own kind more frequently than they cross pair. The *leucocephalus* male usually dominates the hybrid male. This suggests that the differences in the factors affecting sexual isolation between the hybrids and the parent *leucocephalus* are sufficiently small so that the *leucocephalus* male recognizes the hybrid male as a competitor for the females. We are unable to explain why *leucocephalus* males established dominance over hybrid males but not over other *leucocephalus* males. We have obtained no evidence of cross breeding between *leucocephalus* and the hybrids or between *albifrons* and the hybrids under the conditions of the experiment.

Our information about the social behavior of these mice in an unmixed society suggests that differences in social habits are important factors in

TABLE VI

SOCIAL GROUPS FORMED IN AN EXPERIMENTAL SOCIETY OF *PEROMYSCUS*

One pair of a parent stock together with one pair of hybrids in each experiment.

Experiment Number	Total Daily Records	Social Combinations															Litters			Fighting	Analysis
		Double Pairs	Hybrid ♂ with Hybrid ♀	Pure ♂ with Pure ♀	Hybrid ♀ Out	Pure ♀ Out	Pure ♂ Out	Hybrid ♂ Out	None Together	♂♂ Together	♀♀ Together	All Together	♂♂ Together ♀♀ Together	Pure ♂ with Hybrid ♀	Hybrid ♂ with Pure ♀	Double Cross Pairs	Pure × Pure	$F_1 \times F_1$	Father ?		
												leucocephalus with *leucocephalus–blandus* hybrids									
99	32	17	6	......	......	......	1	......	......	......	......	7	......	......	1	......		1		No fighting evident	Definite pairing
48	29	9	1	3	8	2	1	1	......	......	......	......	1	......	2	1			♀L × ?	Both L nearly killed	Definite pairing
101	46	12	2	......	7	......	2	2	......	......	......	20	......	1	......	......				No fighting evident	Together or paired
55	45	5	......	8	16	2	......	4	......	1	2	2	......	3	2	......				No fighting evident	Paired or 2 ♂♂ with ♀L
45	42	3	2	3	5	2	5	4	......	2	......	11	......	2	1	2	1		♀F_1 × ?	No fighting evident	Lived together
43	10	......	......	......	......	......	......	8	......	......	......	......	......	2	......	......				♂ hybrid often bitten	♂L dominant
66	42	7	4	3	......	2	3	7	......	......	2	8	1	5	......	......	2			♂ hybrid often bitten	♂L dominant
104	35	......	1	2	2	2	3	11	......	1	1	1	1	5	2	3		1	♀F_1 × ?	♂ hybrid often bitten	♂L dominant
108	35	6	......	7	......	......	......	14	2	......	......	......	......	5	......	1	1			♂ hybrid often bitten	♂L dominant
Total	316	59	16	26	38	10	15	51	2	4	5	49	3	23	8	7	4	2	3	Some fighting	Paired or ♂L dominant
Expected	316	14.8	29.6	29.6	14.8	14.8	14.8	14.8	29.6	29.6	29.6	4.9	14.8	29.6	29.6	14.8					
												albifrons with *albifrons–blandus* hybrids									
50	32	3	2	1	1	4	6	1	......	......	1	9	......	1	......	3	2			No fighting evident	Lived amicably together
58	34	......	......	......	1	......	6	5	......	......	2	13	2	......	2	3			♀A × ?	No fighting evident	Lived amicably together
109	40	14	3	1	6	2	5	2	......	1	2	3	......	......	1	......				Each ♂ bitten once	Definite pairing
Total	106	17	5	2	8	6	17	8	......	1	5	25	2	1	3	6	2		1	Little fighting	Usually paired or all together
Expected	106	4.7	9.9	9.9	4.7	4.7	4.7	4.7	9.9	9.9	9.9	1.7	4.7	9.9	9.9	4.7					

sexual isolation. Each of the three stocks tested varies markedly in its social behavior. Significantly, the *albifrons* mice, which differ least from *blandus* mice in social habits, are less effectively isolated from *blandus* than are *leucocephalus* mice, which differ the most.

We do not know the actual psychological factors that cause each *leucocephalus, albifrons,* and *blandus* mouse to associate with and interbreed with another of its own stock rather than with an individual of a different but related form. Many factors probably influence the selection of a mate. Physical characters and behavior patterns as well as more elemental stimuli, some perhaps olfactory in nature, probably play a part in the choice of a mating partner. Visual discrimination of physical characters probably is important in the selection of mates. Such discrimination is suggested by the fact that individual *blandus* of the same color strain paired together more frequently than did those of different colors. It is possible, of course, that the mates were selected for characters associated with the pelage color rather than for the color itself.

The sexual isolation between *leucocephalus* and *blandus* and between *albifrons* and *blandus* is not operative in nature, because both *leucocephalus* and *albifrons* are geographically isolated from any representative of the species *maniculatus.* The sexual isolation, however, that exists here would be of the highest evolutionary importance if, as is very possible, either of these forms came to occupy in the future a range overlapping that of *maniculatus.* In that contingency mating preferences would tend to maintain the distinctness of the populations of the two species.

That psychological isolating mechanisms may sometimes break down in nature is indicated by the fact that they can be broken down in the laboratory by allowing no choice of mates. The possible results to be expected of a partial breakdown of the sexual isolating mechanisms are shown by the reactions between the F_1 hybrids and their parent stocks. The *leucocephalus–blandus* and *albifrons–blandus* hybrids pair indiscriminately with pure *blandus,* and at least the *leucocephalus–blandus* hybrids interbreed freely with *blandus.* Thus, if hybridization should occur through the partial breakdown of sexual isolation, the rapid transfer of *leucocephalus* or *albifrons* genes into the *maniculatus* population would be hindered only by the infertility of a fraction of the hybrids.

The barrier to the transfer of *maniculatus* genes into *leucocephalus* or *albifrons,* on the contrary, is lowered but not completely removed by hybridization. Cross pairing in the experiments between the hybrids and the mice of the parent stocks is much less frequent than is pairing between individuals of the respective parent stocks or between the hybrids. Furthermore, so far as we have observed, there is no crossbreeding between the hybrids and the mice of the parent stocks. Therefore, the transfer of *maniculatus* genes into the *albifrons* or *leucocephalus* population would proceed more slowly than the reciprocal transfer, and it might not occur at all.

Should overlapping of their geographic ranges ever occur, it is, therefore, theoretically possible that a modification of the *maniculatus* population by the introduction of foreign genes might take place without any modification of the *albifrons* or *leucocephalus* population through the introduction of *maniculatus* genes. Hybridization, in this case, presumably would not result in the immediate swamping of either kind. Instead, under these conditions some modification of one form (*maniculatus*) would theoretically occur without a reciprocal modification of the other.

It is significant that *leucocephalus* and *albifrons* differ in the degree of their sexual isolation from a representative race (*blandus*) of the species *maniculatus.* Both *leucocephalus* and *albifrons* are geographically isolated populations that W. F. Blair (1943) considers to be incipient species of the cenospecies *maniculatus.* A considerable difference between these two incipient species is indicated by the difference in the extent of their sexual isolation from *maniculatus.* These different degrees of sexual isolation presumably represent different stages in the evolution of their isolating mechanisms.

The existence of psychological barriers to the interbreeding of these geographically isolated populations indicates that sexual isolation develops under conditions of geographical isolation. The evidence here is purely circumstantial. We believe that mice of the taxonomic species *maniculatus* and *polionotus* formerly comprised one interbreeding population that was broken into two arrays during some Pleistocene interglacial period (W. F. Blair, 1943). If this is the true history of these forms, the present aversions to crossbreeding between them must have developed since their geographic separation. It is logical to believe that geographically isolated populations will drift apart in the psychological, physiological, and morphological characters that influence the selection of a mate. The development of sexual isolation probably comes as a byproduct of random divergence in the characters of the two populations. This is in agreement with the hypothesis of Stalker (1942: 255) that "genetic isolating mechanisms would be acquired . . . . during evolutionary changes which result in increased differences between sub-groups."

SUMMARY

Three geographically isolated forms of mice—*Peromyscus maniculatus blandus, P. polionotus albifrons,* and *P. polionotus leucocephalus*—are found to differ markedly in their social behavior when each is tested in an experimental society comprising only mice of its own kind. The *blandus* males are polygamous: one male establishes dominance over the other and monopolizes the company of the females. The *leucocephalus* mice are gregarious: the individuals live harmoniously together. The behavior of *albifrons* is intermediate. Selected color strains of *blandus* tend to pair assortatively.

In the experimental society *leucocephalus* exhibits strong sexual isolation from *blandus.* However, *albifrons* is less strongly isolated from *blandus*

than is *leucocephalus*. The F_1 hybrids between *leucocephalus* and *blandus* and between *albifrons* and *blandus* show no sexual isolation from the parent *blandus*. The hybrids are, on the other hand, sexually isolated from the parent *leucocephalus* or *albifrons*, although this isolation is less effective than that between the pure, parental stocks. It is suggested that if hybridization between these forms should ever take place in nature differential sexual isolation might operate to modify one of the forms without any reciprocal modification of the other.

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